Natural Food Antimicrobial Systems

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26 Sodium chloride

I. INTRODUCTION

Sodium chloride, commonly called salt, table salt, or rock salt, is a vital part of human life. The food that we eat is tasteless without salt. An average American consumes approximately 8-10 g of salt per day (Dickinson, 1980). Salt enhances the flavor of foods and plays a functional role in food processing. For instance, salt controls microbial growth and shifts the fermentation in a desirable direction in products like pickles and sauerkraut; it controls yeast activity, strengthens the gluten and enhances crust color in baking goods; it controls the lactic acid fermentation rate as well as enhances flavor, texture, ripening and shelf life extension in cheese; it lowers water activity, strengthens gel structure and enhances color in processed meats (Lynch, 1987). Apart from its use in the food industry, salt aids in road transportation during severe winter weather, where it is still the best method of ice removal. Salt is also widely used in the chemical industry.

The history of salt dates back to the beginning of civilization, about 2700 BC, when one of the earliest known treatises on pharmacology, published in Chinese, contained information on several types of salt (Salt Institute, 1999). It has been referred to as the essence of life in Biblical teachings (Dickinson, 1980). Salt has been of economical importance in ancient history. In ancient Greece, goods were exchanged for salt. Salt tax has been an important issue of human history in many parts of the world. In Chinese history the salt tax was a major source of revenue (Salt Institute, 1999). Salt was a cause of the French Revolution. The salt tax supported British monarchs and people were imprisoned for smuggling salt. During the British rule in India, Mahatma Gandhi opposed the salt tax during the struggle for freedom. The desire for salt is believed to be an evolutionary process (Forsyth & Miller, 1980). It has been suggested that the neural-endocrine interactions in higher mammals play a role in behavior towards salt emergence and salinity control, and this relates to their parallel evolution (Denton, 1982). Studies on kangaroos living on the snowy mountains of Australia show a specific sodium appetite organized in the animals' brains (Denton, 1982).

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Other related compounds of sodium that are used in food processing and preservation include sodium ascorbate, sodium benzoate, sodium bicarbonate, sodium citrate, sodium nitrate, sodium nitrite, sodium phosphate and sodium propionate. Some other salts that have been tried as a substitute for sodium chloride include potassium chloride, calcium chloride, ammonium chloride, magnesium chloride and lithium chloride. Each of these has limitations. Potassium chloride is the most closely related compound to sodium chloride, especially with regard to its physical and functional properties, but the limiting factor is its extreme bitter taste. Calcium and magnesium chlorides are very bitter too. Ammonium chloride is a highly unstable compound and lithium chloride is toxic to humans.

Replacing sodium chloride partially with a mixture of potassium, magnesium and calcium chlorides in dry fermented sausage caused a decrease in saltiness (decrease in sensory acceptability) as well as an increase in acidity and water activity (Gimeno et al, 1998). The lactic acid bacteria were not affected, but the counts of Micrococcaceae decreased. However, partial replacement of sodium chloride with potassium lactate in dry fermented sausages did not cause significant sensory defects (Gou et al., 1996; Askar et al., 1993). Partial replacement of sodium chloride with magnesium chloride at low levels did not produce any sensorial defects in bologna (Seman et al., 1980) while in frankfurters magnesium chloride was not a suitable substitute (Hand et al., 1982). Substituting sodium chloride with glycine (above 40%) produced an unacceptable sweet taste and non-uniform texture (Gou et al., 1996). Potassium chloride has been used as a substitute in some formulations (Ibanez et al., 1995; 1996; 1997) while substitution of the same has caused significant bitterness in some others (Leak et al., 1987; Keeton, 1984). A 1:1 mixture of sodium and potassium chloride when fed to human subjects showed a reduction of sodium intake by 44-55% (Mickelson et al., 1972).

Several seasonings and flavoring blends are also being tried as substitutes for sodium chloride. One such substitute included a mixture of potassium chloride, hydrolyzed vegetable protein, dipotassium orthophosphate, glucose, 5'-inosinic acid and 5'-guanosinic acid (Mohlenkamp & Miller, 1981). This blend was free of the bitter taste associated with potassium chloride. Autolyzed yeast blended with potassium chloride reduced the bitter taste due to potassium chloride (Shackelford, 1981). Another low sodium seasoning blend consisted of potassium chloride, monopotassium glutamate, potassium citrate, potassium phosphate, L-glutamic acid and silicon dioxide (Allen & Day, 1980). Glycinamide hydrochloride formed a good blend with monosodium glutamate (Sternberg et al., 1980). Addition of certain amino acids and their esters with sodium chloride has been found to enhance saltiness. L- ornithyl-B-alanine and Glycine ethyl ester hydrochloride enhanced the salty effect of sodium chloride (Okai et al., 1989). Some of the salty peptides included glycine methyl, ethyl ester hydrochlorides (Kawasaki et al., 1988) and L-ornithyltaurine (Tada et al., 1984).

II. OCCURRENCE AND PROPERTIES

Salt is abundantly available in nature and has been designated as the fifth element, next to air, water, fire and earth (Dickinson, 1980). In the United States, salt deposits are found in the states of Michigan, Ohio, New York, Pennsylvania, Louisiana and Texas. The country has an annual production of about 22 millions of dry salt (Dickinson, 1980). Historically, salt was made by boiling brine from salt springs. In 1800,

large-scale production of salt began and the first underground salt mine was found in 1869 (Salt Institute, 1999).

Salt is produced by three methods (Dickinson, 1980). Recovering the rock salt by digging 600 to 2000 feet below the surface of the earth is called mining. Explosives are used to loosen the salt embedded in the rocks in the mines. Another method is removing the brine from brine wells using pipes, wherein, a pipe flushes water into a salt deposit and the brine formed is removed and brought to the surface using another pipe. The water is then evaporated or removed by heat, which leaves salt granules or flakes. The third method is natural evaporation of water from concentrated brines by sunshine, producing solar salt. The brine is concentrated in ponds to a point where it crystallizes and evaporates to form a salt bed. The crystallized salt is then harvested, washed and screened. This method can be only used in areas of heavy sunshine, very little rain and good air motion. In the United States, the sources of solar salt include the great Salt Lake in Utah, as well as the San Francisco Bay and San Diego areas in California.

Salt as a chemical compound is made of two elemental substances, the cationic sodium ion (Na^+) and the anionic chloride ion (Cl^-), which react together forming the halide salt, sodium chloride. The role of sodium ion in the human body is to maintain the blood volume and cellular osmotic pressure as well as aiding in the transmission of nerve impulses (Institute of Food Technologists, 1980). With regard to the physical properties of sodium chloride, it is present either as colorless transparent crystals or as white crystalline powder, with a molecular weight of 58.44, melting point of 80°C, boiling point of 1413°C, pH of 6.7 to 7.3 and a density of 2.165 (Lewis, 1980). It is soluble in water and glycerine. One gram of sodium chloride will dissolve in 2.8 ml of water at 25°C and a saturated solution will contain 26.5 g sodium chloride in 100 g of water (Shelef & Seiter, 1993). The solution is clear, colorless and odorless. It is stable under ordinary conditions of use and storage. Sodium constitutes 40% of sodium chloride by weight (Bodyfelt, 1982). To determine the salt content of a product, the food or its ash is extracted in warm water and the sodium and chloride contents determined by appropriate techniques. The concentration of sodium present in various bodily secretions can be measured through either flame photometry or by ion-specific electrodes (Michell, 1995). The chloride can be measured by titration using Morh's or Volhards's method (Luick, 1980). For assays of sodium chloride in various foods refer to 'Official Methods of Analysis' of the American Association of Analytical Chemists (1990).

III. ANTIMICROBIAL ACTIVITY

The antimicrobial activity of sodium chloride may be called either direct or indirect depending upon the purpose it serves and the amount added in a food product (Sofos, 1983). In case of dried and smoked meats, a large amount of sodium chloride is added, which makes these products shelf stable. These products were popular in earlier days and in certain parts of the world. They depended solely on sodium chloride for their preservation, and hence the effect could be called direct. In recent years sodium chloride is added in minimal amounts and is combined with other preservatives or hurdles to prevent microbial growth. The amount of sodium chloride that needs to be added in foods required to prevent microbial growth is large (16.54% salt solution to bring the water activity to 0.90 (Robinson & Stokes, 1959)) and will cause an unacceptable taste and hence salt is usually combined with other preservation techniques. In certain instances, sodium chloride is

added mainly as a flavoring and functional ingredient and hence in these cases the effect could be called indirect. In some fermented food products, sodium chloride in appropriate concentrations extracts nutrients from vegetables, which in turn allows the growth of lactic acid bacteria, thereby preventing growth of certain spoilage organisms and even pathogens such as *Staphylococcus* species. In these cases, the antimicrobial effect could be called indirect. Another reason that the antimicrobial effect of sodium chloride may be called indirect is that it reduces the water activity in many foods (Sperber, 1983) and thereby indirectly prevents microbial growth. The reduction in water activity of the food causes an osmotic shock to the bacterial cell. Plasmolysis occurs and the cell loses fluids and either dies or enters dormancy. Some other possible mechanisms of sodium chloride inhibition are limiting oxygen solubility to the microbial cell, alteration of pH, toxicity of sodium and chloride ions, loss of magnesium ions (Banwart, 1979) and interference with the cellular enzymes (Shelef & Seiter, 1993).

Studies on the effect of sodium chloride on various organisms have indicated that sodium chloride could have a role on interfering with substrate utilization in these organisms. In *Staphylococcus aureus* sodium chloride was found to inhibit respiration, glucose utilization, phospho-β-galactosidase induction, and staphylococcal enterotoxin-A synthesis as well as hydrolysis of *O*-nitrophenyl-β-galactoside (ONPG), thereby inhibiting substrate transport into the bacterial cell (Smith et al., 1987). Erecinska and Deutsch (1985) studied the effect of sodium chloride on washed cells of *Paracoccus denitrificans* and found that increasing the concentration of sodium chloride (from 0.2 to 1.8%) gradually decreased the respiration rate and uptake of α-isoaminobutyrate with the highest concentration, completely inhibiting the uptake of the amino acid. In *Clostridium sporogenes*, glucose utilization was gradually inhibited and the level of intracellular ATP progressively decreased with increasing concentration of sodium chloride (Woods & Wood, 1982). However, the degree of glucose conversion to ethanol remained unchanged, indicating that sodium chloride interfered only with uptake and not with metabolism. In *Pseudomonas fluorescens* an increasing concentration of sodium chloride inhibited oxygen uptake (Prior, 1978). Protein synthesis was not always inhibited in different *S. aureus* cultures (Troller and Stinson, 1978).

Bacteria can be classified into different groups based on their salt tolerance (Ayers et al., 1980). Those that grow well at concentrations ranging from 0 to 0.5% as well as tolerating higher levels are called nonhalophiles. Some bacteria belonging to the genera *Staphylococcus*, *Micrococcus* and *Clostridium* belong to this group. Those that can tolerate and grow in the range of 1.5 to 5.0% sodium chloride are called slight halophiles and some members of *Achromobacter*, *Flavobacterium*, *Pseudomonas* and *Vibrio* fall into this category. The third group, which includes those that can tolerate up to 5 to 20% sodium chloride, are called moderate halophiles. Some of the lactic acid bacteria and some spore formers fall under this category. Some of the moderate halophiles were studied for their susceptibility to a wide range of antibiotics at different salt concentrations and a group of them were found to have increased susceptibility at low salt concentrations (Coronado et al., 1995). Organisms that require and can grow in high concentrations of salt are called halophiles and those that can tolerate but not grow in high concentrations of salt are termed halodurics (Jay, 1992). The extreme halophiles require at least 9% sodium chloride for growth, with the optimum concentration for growth ranging between 12 to 23% and the maximum being 32% (Madigan et al., 1997). The cell wall of halophilic strain *Halobacterium salinarum* is stabilized by sodium ions, to such an extent that in low

sodium environments or where the concentration of sodium is insufficient the cell wall breaks down causing cell lysis (Madigan et al., 1997). The need for sodium cannot be replaced by any other ion, including potassium. Obligate halophiles require and tolerate 15% sodium chloride and can be found in saturated brines. Bacteria that can grow in high salt concentrations however, are of less importance as food spoilage or pathogenic agents (Lueck, 1980).

Sufficiently high amounts of sodium chloride are needed to cause bactericidal effects in foods and such amounts would not be permissible in foods, and such foods may not even be palatable. Hence, sodium chloride is generally used with other preservation methods. The antimicrobial effect of sodium chloride is thus dependent upon on several other factors present in foods as well as during food processing. Some of these factors include pH of the food, temperature and time during processing and storage, type of substrate, water activity of the substrate, type and populations of microorganisms, other preservatives/antimicrobial present in the substrate etc. Hence, most of the research done on sodium chloride is on its combinations with other hurdles and these studies will be discussed.

As mentioned earlier, the mechanism of salt inhibition is by lowering the water activity of the substrate. Most of the pathogenic organisms will not grow below water activity of 0.90 to 0.92. One exception is *Staphylococcus aureus*, which has a generally recognized minimum water activity of 0.86, but has been reported to grow in conditions of water activity as low as 0.83 (Jay, 1992). This organism can grow in the presence of 7 to 10% sodium chloride with some strains being able to grow at 20% sodium chloride. Concentrations of sodium chloride up to 10% had very slight effects on the growth of *S. aureus*, but concentrations more than 3% caused a decline in the production of enterotoxin-B (McLean et al., 1968). In another study, both the cell growth and enterotoxin-B production were inhibited with increasing sodium concentration (0 to 5.3%), but there was no effect on enterotoxin-A production (Troller & Stinson, 1978). However, other researchers have shown an inhibitory effect of sodium chloride on enterotoxin-A production by *S. aureus*. Increasing the concentration from 0 to 4% (Pereira et al., 1982) and from 0 to about 10% (Smith et al., 1987) caused a decline in the enterotoxin-A production by the organism. An increase in the concentration of sodium chloride in tryptic soy broth caused an increase in the effectiveness of butylated hydroxyanisole (BHA) in inhibiting *S. aureus*. Accordingly, the strongest effect was observed at a combination of 100 ppm BHA and 5% or 10% sodium chloride (Stern et al., 1979). Sodium chloride (7%) exhibited a synergistic effect with potassium sorbate (0.2%) in inhibiting *S. aureus* strains in TSB at 37°C (Robach & Stateler, 1980). However, there was no synergistic effect of sodium chloride (2, 3 and 7%) and potassium sorbate (0.2 and 0.3%) observed after prolonged exposure (15 days at 22°C and 48 h at 35°C) (LaRocco & Martin, 1987). An alternative sigma factor s^B, produced by *S. aureus* as a response to environmental stresses was repressed in the presence of 1 M sodium chloride (Chan et al., 1998).

There are reports in the literature showing that the heat resistance of *S. aureus* increases with increasing concentrations of sodium chloride due to a decrease in the water activity of the medium. Sodium chloride at different concentrations (3, 5 and 9 % w/v) protected *S. aureus* from heat injury, with the highest concentration affording the maximum protection (Smith et al., 1982). The authors suggested that sodium chloride might be involved in stabilizing membrane protein structures such as nucleic acids and nucleotides as well as increasing the melting temperatures of membrane phospholipids.

Thus, the damage to the cell membrane and leakage of cell components from the cytoplasm is prevented. In another study, 4 and 8% sodium chloride protected *S. aureus* cells from heat injury at pH 7.0 while at pH 6.5 a concentration of 8% gave protection (Bean & Roberts, 1975). Different substrates with reduced water activity were inoculated with *S. aureus* and the organism showed increased heat resistance (Troller, 1973). The effects of salt on thermal resistance have been examined by determining the relationships between thermal resistance and either solute concentration or water activity of the heating menstruum (Juneja et al., 1999). The heat resistance of *S. aureus* in sodium chloride increased as the degree of salt-water association increased (Tuncan & Martin, 1990). The authors explained that the effects of salt on thermal inactivation of microorganisms are mainly related to reduced water activity and increased osmotic pressure of the heating menstruum. For a given solute a certain optimum concentration of the solute gives a maximum heat protection, whereas levels outside this optimum solute concentration result in an increase in the heat sensitivity of the organism (Leistner & Russell, 1991).

The influence of sodium chloride on spore formers has been the subject of interest for many researchers. Sodium chloride has been found to inhibit toxin production in *Clostridium botulinum.* Greenberg et al. (1959) studied the inhibitory effect of sodium chloride on growth and toxin production in *C. botulinum* types A and B in cured meat. With less than 6.25% sodium chloride there was no inhibition of toxin production as well as putrefactive changes. Between 6.25 and 9% there was no inhibition on toxin production but the putrefactive changes did not occur. Above 9% growth was inhibited. In laboratory medium, 3 to 4 % sodium chloride inhibited toxin production by *C. botulinum* type E (Segner et al., 1966). The influence of sodium chloride on toxin production by *C. botulinum* types A, B and C at different temperatures in cooked meats vacuum packed in air-impermeable plastic pouches was studied (Pivnick & Barnett, 1965). Toxin production in both types was inhibited by 3% salt during 4 weeks at 30°C. However, 2.2% salt did not inhibit toxin production at 30°C in 1 week, while it did in 1-month at 25°C and in 2 months duration at 20°C. Sodium chloride at concentrations 2.1 and 3.4% inhibited toxin production in cooked ham within a month at 15°C. In the case of *C. botulinum* type E, 1.9% sodium chloride failed to inhibit toxin production in jellied pork tongue, while 2.7% sodium chloride was very effective in inhibiting toxin production. The authors suggested adequate refrigeration and an increase in salt concentration to acceptable limits for types A and B and heat and low salt concentration for type E. Sodium chloride at 1.4 and 2.3% concentrations did not inhibit toxin production by *C. botulinum* E in jellied ox tongue (Pivnick & Bird, 1965). A concentration of 5.0% sodium chloride at pH values lower than 5.03 was needed to inhibit growth of *C. botulinum* type E (Segner et al., 1966).

The effect of sodium chloride on growth of *C. botulinum* spores at different temperatures and pH levels in the presence of sodium nitrite was investigated (Emodi & Lechowich, 1969). The substrate was laboratory media and the pH range varied from 5.2 to 6.6. About 4.87% sodium chloride was sufficient to inhibit outgrowth at all temperatures (30, 15.6, 10, 7.2, 5.0 and 3.4) with lower temperatures requiring slightly lower concentrations. At lower temperatures, inhibition occurred at high pH levels. In bottled lumpfish caviar toxin production of *C. botulinum* was inhibited at combinations of sodium chloride concentrations $\geq$5.56% and pH <5.0 at an abusive temperature of 30°C (Hauschild & Hilsheimer, 1979). Products with 4.5% sodium chloride were capable of supporting growth of proteolytic *C. botulinum* strains even at 15°C, even though they may have an extended lag time (Gibson et al., 1987).

The effect of sodium chloride on the heat resistance and recovery of non-proteolytic *C. botulinum* type B strain in turkey meat slurry was studied (Juneja & Eblen, 1995). Increasing the salt concentration from 1 to 2 and 3% reduced the heat resistance of the spores (the spore D-values declined). The measured heat resistance in the heating medium as well as the recovery medium (reduced recovery of heat-injured spores) was lower with increasing salt concentrations. A similar phenomenon of reduction in recovery with increasing salt concentrations has been observed in other spore formers such as proteolytic *C. botulinum* (Pivnick & Thacker, 1970), *C. sporogenes* (Roberts et al., 1966) and *Bacillus stearothermophilus* (Cook & Gilbert, 1969). Interactive effects of temperature, pH, sodium chloride and phosphate on the thermal inactivation of non-proteolytic *C. botulinum* type B strains have been studied (Juneja et al., 1995). Combined with heat treatment sodium chloride was able to prevent growth from spores of non-proteolytic *C. botulinum*. (Stringer & Peck, 1997). Heating at 90°C for 30 min alone or incubating at 10°C with 4.0% salt by itself did not prevent growth, while heat treatment at the same conditions following by incubation with the same amount of salt did prevent growth. Sodium chloride at 3% concentration along with low pH (5.5) reduced the nisin resistance of *C. botulinum* strains (Mazzotta et al., 1997).

The effect of sodium chloride on spore outgrowth of *C. perfringens* in cook-in-bag ground beef at different temperatures, pH and sodium pyrophosphate levels was investigated (Juneja & Majka, 1995). At 28°C, a combination of 3% sodium chloride and pH 5.5 delayed growth for 24 h. At 15°C and pH 7.0 there was growth within 6 days, but with the same conditions when 3% salt was added growth was delayed until 8 days. At pH 5.5 and 15°C in the presence of 3% salt, there was no growth of vegetative cells even after 21 days. The temperature abuse of this product for more than 15 h without sodium chloride led to growth of *C. perfringens*. In case of vacuum packaged cook-in-bag ground turkey, at 28°C 3% salt delayed growth of *C. perfringens* from spore inoculum for 12 h (Juneja & Marmer, 1996). At 15°C with 1-2% salt growth was very slow. With 3% salt no vegetative cells were observed even after 28 days. The D-value was 23.2 min with no salt, which decreased to 17.7 min with 3% salt. Gibson and Roberts (1986a) studied the influence of increasing concentration of salt in combination with sodium nitrite, pH and temperature variables, and found that the growth rate of *C. perfringens* decreased with increasing concentrations of salt. Salt showed synergistic effects with other variables. Juneja et al. (1996) studied the interactive effects of sodium chloride, temperature, initial pH and sodium pyrophosphate on growth kinetics of *C. perfringens*.

The effect of sodium chloride on heat and radiation resistance and on recovery of injured spores of *Bacillus* species was investigated (Briggs & Yazdany, 1970). An increase in the concentration of sodium chloride caused a decrease in the heat resistance of *B. sterothermophilus*, but not the radiation resistance of the same organism as well as the heat and radiation resistance of other species of *Bacillus*. Sodium chloride presence during heating increased the sodium chloride sensitivity of *B. stearothermophilus*, but not that of other species. An increase in the irradiation dose increased sodium chloride sensitivity of *B. stearothermophilus* in the recovery medium while the effect was less severe for other species. The growth of *B. stearothermophilus* in salty carrot medium as a function of pH, temperature and sodium chloride has been characterized and mathematical models derived (Ng & Schaffner, 1997). *B. subtilis* when exposed to mild concentrations of salt was able to survive subsequent toxic concentrations (Volker et al., 1992). A mild heat shock produced cross-protection against lethal salt stress, but not vice-versa. A set of general stress proteins were believed to be induced in response to salt or heat stress.

The effect of sodium chloride in combination with other factors on *Escherichia coli* has been investigated. A concentration of 8% or more of sodium chloride completely inhibited growth of enteropathogenic *E. coli* at different temperature and pH levels, while a concentration of 4% in combination with pH 5.6 and 200 ppm of nitrite did not (Gibson & Roberts, 1986b). *E. coli* O157:H7 was inhibited by 8.5% or more sodium chloride in TSB (Glass et al., 1992). A concentration of 2.5% did not have any inhibiting effect, while at 4.5% the generation time was longer and at 6.5% the lag time was very long (36 h) which the authors attributed to the presence of a salt tolerant population of the organism. In fermented sausage with 3.5% sodium chloride, 69 ppm. sodium nitrite and pH 4.8 the organism population was reduced but was not inhibited completely (Glass et al., 1992). The survival of bioluminescent *E. coli* O157:H7 in brain heart infusion medium (BHI) containing sodium chloride with other factors as well as in model systems representing fermented sausage was studied (Tomicka et al., 1997). Sodium chloride at concentrations up to 3.5% did not inhibit growth in BHI. In American-style fermentation (high temperature short time) with 2% sodium chloride, starter culture, dextrose and sodium nitrite, the organism survived for more than 51 days and the authors explained that sodium chloride and sodium nitrite enhanced the survival of the organism in this system. In the European-style fermentation, at low inoculum levels of the organism there was inhibition after 9 days, while with high initial inoculum levels, there was survival for more than 30 days. The reason for this survival was attributed to possible inhibition of starter cultures by the salts added and hence lesser competition for *E. coli*. In skim milk (10% rehydrated non-fat dry milk), *E. coli* O157:H7 had up to a 3-log reduction with 4% salt at pH 4.7 at different times and temperatures, while 6% sodium chloride caused complete inhibition at all treatment conditions (Guraya & Frank, 1998). It was concluded that sodium chloride at increasing concentration enhanced inactivation at pH levels between 4.1 and 4.7.

The interactive effects of temperature (55 to 62.5 °C), pH (4 to 8), sodium chloride (0 to 6% w/v) and sodium pyrophosphate (0 to 0.3% w/v) on the heat resisnce of *E. coli* O157:H7 was examined (Juneja et al., 1999). All four factors interactively affected the inactivation of the pathogen, and a mathematical model for the interactive effects of the four variables on the thermal inactivation was developed. The protective effect of salt against lethality was only observed at pH 4.0. The influence of sodium chloride (8%) combined with sodium lactate (4.0%) and polyphosphate (0.5%) on the heat resistance of turkey meat at 50, 55, 57 and 60°C was studied (Kotrola & Conner, 1997). The D- values for turkey meat with these additives were higher than turkey meat without additives, indicating that the additives enhanced survival of the organism. A predictive model fitted using the Gompertz equation for the growth of *E. coli* O157:H7 as a function of temperature, pH and sodium chloride was developed by Sutherland et al. (1995) and has been extrapolated to a range of foods including meat, poultry, milk, cheese and tempeh. Acid adaptation of some strains of *E. coli* O157:H7 exhibited cross-protection against increased osmolarity (Garren et al., 1998; Cheville et al., 1996). Salt, heat and acid tolerances in *E. coli* O157:H7 were found to be regulated by the *rpo*S sigma factor (Cheville et al., 1996).

Another organism studied with regard to its sensitivity to sodium chloride is *Listeria monocytogenes*. The influence of sodium chloride, temperature and pH on the growth of *L. monocytogenes* in cabbage juice was investigated (Conner et al., 1986). A 2% and higher concentration of sodium chloride in the juice inhibited the organism. With 5% sodium chloride the survival of one strain (Scott A) declined 90% over 70 days at 5°C. Another strain (coleslaw outbreak strain) survived in cabbage juice containing 3.5% or

less sodium chloride over a 70 day period. At 1.5 and 2.0% sodium chloride, both the strains had extended lag times. Initial exposure to heat and ethanol significantly increased the tolerance of *L. monocytogenes* to sodium chloride (Lou & Yousef, 1997). Sodium chloride inhibited the repair of thermal injury in *L. monocytogenes* cells (Pagan et al., 1997; Linton et al., 1992; Smith & Hunter, 1988; Golden et al., 1988).

Models to fit the interactive effects of sodium chloride with other variables on *L monocytogenes* have been the subject of interest for many investigators. The heat resistance of *L. monocytogenes* at different temperatures, pH levels and sodium chloride concentrations was studied in phosphate buffer (Linton et al., 1995) and in infant formula (Linton et al., 1996) and models were fit using the Gompertz equation. Interactive effects of temperature, lactic acid, sodium chloride and sodium nitrite on the survival of *L. monocytogens* under anaerobic conditions (Buchanan & Golden, 1995) and the interactive effects of these same factors on the time to achieve a 4-log reduction in aerobic acidic conditions (Buchanan et al., 1997) were studied and models fit for the same. Another model included the effect of CO_2, pH, temperature and sodium chloride on the growth of *L. monocytogenes* (Fernandez et al., 1997). Juneja and Eblen (1999) studied the interactive effects of temperature, pH, sodium chloride and sodium pyrophosphate on the heat inactivation of *L. monocytogenes* in beef gravy. In their findings, sodium chloride protected the organism against the lethal effects of heat. A predictive model describing the combined effects of the factors was developed. Another model describing the effects and interactions of temperature, pH, growth atmosphere (aerobic and anaerobic), sodium chloride and sodium nitrite on the growth of *L. monocytogenes* was developed by Buchanan and coworkers (1989).

Effects of sodium chloride on other food-borne pathogens have also been documented. Thermally processed as well as fresh food products are chilled using brine that could contain up to 20% sodium chloride. The growth, survival and injury potential of *Yersinia enterocolitica*, *S. aureus* and *L. monocytogenes* in chiller brine with different sodium chloride concentrations was investigated (Miller et al., 1997). Growth of *Y. enterocolitica* was observed in 0.5 and 5% brine at -2 and 5°C, respectively. *S. aureus* grew in 5% sodium chloride at 12°C and *L. monocytogenes* was able to grow in 5 and 9% sodium chloride at 5 and 12°C, respectively. Sodium chloride (9%) at -2°C was either bacteriostatic or -cidal depending on the organism. *L. monocytogenes* was able to survive 20% sodium chloride at -12°C for 30 days. Caution should be taken in recycling brine, if such survival could occur.

Li et al. (1997) tested the efficacy of sodium chloride spray to reduce *Salmonella typhimurim* in prechill chicken carcasses. Prechill chicken carcasses inoculated with *S. typhimurium* were sprayed with tap water, 0.85% sodium chloride solution and other antimicrobial sprays. Compared to tap water, sodium chloride did not significantly reduce *S. typhimurium* population and hence did not prove as effective an antimicrobial spray. The efficacy of sodium chloride and sodium carbonate solutions on removing *E. coli* O157:H7 from surfaces of chopped lettuce was studied (Janes et al., 1999). A 2-log reduction was observed with a concentration lower than 0.8% and about 2.5-log reduction with 0.8 to 1% solutions of each of these salts at pH 8.0, whereas a 3-log reduction was observed at pH 2.0 and 10. *Shigella* is a foodborne pathogen of concern that can survive in seawater (Nakamura et al., 1964). *Shigella flexneri* strains were able to grow in 3.78% sodium chloride (highest concentration allowing growth in nutrient agar) at 37°C (Fehlhaber, 1981). The interactive effects of sodium chloride, pH and temperature on the

growth of *S. flexneri* was examined (Zaika et al., 1989). Combinations of low temperature, low pH and high sodium chloride concentration proved inhibitory to the organism. The inhibitory effect of a combination of antimicrobial compounds (lysozyme, monolaurin, triglycerol 1,2 laurate and BHA) against a number of spoilage and pathogenic microroganisms (*Bacilus* sp., *Pseudomonos* sp., *Enterobacter aerogenes, Lactococcus lactis, L. monocytogenes, E. coli* O157:H7, *Staphylococcus* sp., *S. typhimurium*) in the presence of sodium chloride and EDTA was studied (Razavi-Rohani & Griffiths, 1996). Sodium chloride exhibited a synergistic effect with these combinations in the presence of EDTA and low pH in inhibiting the organisms. The effect of sodium chloride and nitrite on the antimicrobial activity of lysozyme, nisin, and EDTA combination treatments against a variety of microroganisms of concern in cured meats was tested (Gill & Holley, 1999). Sodium chloride alone was inhibitory against *E. coli* O157:H7, *Salmonella typhimurium, Serratia grimesii* and *Shewanella putrifaciens*. A combination of sodium chloride and EDTA was found to be inhibitory to *E. coli, Salmonella* and *Serratia*, while in combination with sodium nitrite, sodium chloride inhibited *Salmonella* and *Shewanella*. Water containing electrolytic products of sodium chloride (electrolytic water) was tested for its bactericidal and hand washing efficiency and compared with hypochlorite solution (Hitomi et al., 1998). The electrolytic water was equally effective in its bactericidal and handwashing efficacy and is recommended for handwashing in place of running water.

IV. BIOLOGICAL ADVANTAGE

Sodium is a nutrient essential for all animals including humans. It is a dominant ion of human extracellular body fluid both quantitatively and functionally and is important for maintaining an appropriate blood volume and pressure, cell osmotic pressure and for transmitting nervous impulses (Institute of Food Technologists, 1980). Both sodium and chloride ions predominate in the extracellular body fluid whereas in the intracellular body fluid, sodium is present in very small quantities with almost no chloride present. Chloride is essential for maintaining tissue osmolarity, acid-base balance in the blood and the electrolyte balance of the body, for activating some of the essential enzymes in the stomach and for the formation of hydrochloric acid in the stomach, for transmitting nervous impulses and for the passage of water across the cell walls (Schreiber & Harner, 1983; Institute of Food Technologists, 1980). According to Schreiber and Harner (1983) the physiological roles of sodium chloride in the human body include: i) regulation of extracellular fluid volume. A decrease or increase in the bodily sodium chloride will cause a corresponding change (increase or decrease) in the extracellular body fluid. ii) regulation of intracellular fluid. Based on the amount of salt the intracellular water maintains equilibrium with the extracellular body fluid. iii) regulation of neutrality of body fluids. The body fluids have a neutral pH and their neutrality is maintained by sodium buffer systems and the excretory action of kidneys and lungs. iv) other physiological processes such as cardiovascular, intestinal muscles and the stomach require exact proportions of sodium and chloride. Any of these ions if excessively consumed, the amount other than needed will be excreted by the body in the urine and a balance between the two ions is always maintained by the body unless there are other disorders. Sodium chloride has its benefits in the clinical field. Intra-dermal bacteriostatic sodium chloride (0.9%) containing the

TABLE 1. Sodium content of some natural and processed foods

Food product	Sodium content (mg/100g)
Celery	140
Carrot	50
Apple	2
Milk	50
Lightly salted butter	870
Stilton cheese	1150
White bread	540
Soy sauce	6082
Tomato ketchup	1120
Boiled eggs	140
Corned beef	950
Steak	54

Adapted from Paul & Southgate (1978)

preservative benzyl alcohol was found to be as effective as 1% lidocaine hydrochloride (an amide local anasthetic) for the attenuation of intravenous cannulation pain (McNelis, 1998).

The minimum daily requirement of sodium chloride for normal individuals is less than 2 g/day and the recommendation of health agencies is not to exceed 6 g/day. The daily dietary intake of sodium is estimated to be 1.1 and 3.3 g for adults and between 115 and 750 mg for infants (NAS, NRC, 1980). For chloride, the minimum requirement daily set by the food and nutrition board is 750 mg for adults and 180 to 350 mg for infants. There are several sources through which sodium chloride is consumed by humans. The potable water contains sodium chloride that accounts for less than 1% of the daily intake. Sodium chloride is also present in some natural food products such as fruits and vegetables. Another source of salt is many processed foods. The sodium content of some natural and processed foods is summarized in *TABLE 1*. Another major source of sodium chloride is the salt added during cooking or at the dining table. Drugs (medicines) taken for ailments may also contain sodium and hence people who are taking medications regularly or for ailments should consider such intake. Excessive sodium chloride intake has been identified to be one of the causes for developing hypertension and the related cardiovascular problems and stroke (Pearson & Wolzak, 1982), however, a clear link between the two has not been established. Because of such possible health risk, there are many consumer concerns and the food industry is trying to minimize the salt content of food products.

V. APPLICATIONS

Salt is used not only by the food industry but also by the agricultural, chemical and transportation industries. *TABLE 2* summarizes the average amount and cost of salt by the various industries (adapted from Dickinson, 1980). In many food formulations salt is added for flavoring, functional as well as preservative effects. Some of these food products include butter, cheese, fermented vegetables and fish, cured meats, bread etc. In butter and margarine, salt is used as a flavoring agent but also serves as a preservative. In these products, emulsified fats in the water phase are susceptible to microorganisms. Up to 2% and 3% by weight of salt is added in butter and margarine respectively after wash-

TABLE 2. Average amount of salt used and the cost incurred by various industries in the US

Industry	Amount of salt used (tons)	Cost incurred ($)
Food	1,002,800	98,302,900
Chemical	4,114,200	37,450,000
Highway	10,927,000	97,779,600
Water conditioning	2,304,800	78,947,000
Agriculture	1,980,000	73,944,300
TOTAL	20,328,800	386,423,800

Adapted from Dickinson (1980)

ing the grains before kneading (Lueck, 1980). The addition of 1-4% of salt to butter causes a significant increase in shelf life (Schreiber & Harner, 1983). There is about 16% moisture in these products, which allows for up to 12.5% sodium chloride, the concentration that can be inhibitory to most microorganisms at refrigeration temperatures (Shelef & Seiter, 1993).

In the case of cheeses, salt acts as a flavoring and functional ingredient as well as helping preservation when combined with other antimicrobials such as sorbates. Salt is added to cheese curd either in the dry form or as a solution, based on the type of cheese. Up to 5% salt, relative to the water content of cheese is considered optimum (Lueck, 1980). Salt inhibits the development of bitter flavor in cheddar cheese by inhibiting the proteolysis of β-casein (Fox & Walley, 1971). Sodium chloride introduced in the serum phase of mozzarella cheese, promoted microstructure swelling, caused an increase in water holding capacity, and promoted solubilization of intact caseins from the paracasein matrix (Guo et al., 1997). Sodium chloride seems to play a role aiding/affecting the functional properties of some proteins and carbohydrates. Addition of sodium chloride to process whey protein solution increased the viscosity and aided in gelation (Kinekawa et al., 1998). Sodium chloride inhibited the *in vitro* proteolysis/digestibility of phaseolin proteins from dry beans such as the Great Northern bean and tepary bean (Sathe & Sze-tao, 1997; Sathe et al., 1994; Sathe et al., 1984). Sodium chloride aided in the clarity of starch and amylopectin (amaranth, waxy corn, normal corn) pastes, with the % transmittance values increasing with increasing salt concentration (Bello-Perez & Paredes-Lopez, 1996). Sodium chloride induces aggregation and gelation of some polysaccharides. 200 mM sodium chloride aided carageenan to form a strong gel, while in the presence of 100 mM salt a weak gel was formed with a mixture of carageenan and locust bean gum (Goncalves et al, 1997).

In fish, salting has been one of the oldest methods of preservation. Soon after harvest, the fish are cleaned and salt is added. Fish is one of the commodities to which a large amount of salt is sometimes added and if lesser salt is added, it is usually combined with other methods of preservation. Dipping fish in sodium chloride solution preserves the texture and color combined with modified atmospheric packaging (MAP) and storage (Mitsuda et al., 1980). Hake slices were dipped in sodium chloride solution (5 min in 5% brine) and MAP stored and these were compared with MAP stored slices of hake without sodium chloride dipping (Pastoriza et al., 1998). In sodium chloride dipped slices, biochemical, microbiological and sensory deterioration changes were inhibited, shelf life extended and the total volatile bases and total viable microbial counts were significantly lower than those of non dipped slices. The postmortem changes (rigor mortis) of Atlantic salmon influenced the salt uptake of the fish muscle (Wang et al., 1998). The equilibrium

salt concentration of pre-rigor fillet was much lower (0.53 g/g salt-free solids) than that of in-rigor (0.66 g/g salt-free solids) and post-rigor mortis (0.75 g/g salt-free solids) salmon fillets in 20% (w/v) sodium chloride solution at 10°C.

In the case of liquid whole egg and egg yolk, 5 to 8 % of sodium chloride is used for preservation. Sodium chloride had a cryoprotective effect on egg yolk stored at -24°C by inhibiting its gelation at concentrations of 4 to 8% but not at 10% (Telis & Kieckbusch, 1998). Sodium chloride at concentrations of 0.1 and 1.0 M and at pH 7.2 and 9.0 protected egg white lysozyme, a natural antimicrobial enzyme, against heat inactivation at temperatures between 73-100°C (Makki & Durance, 1996). Sodium chloride was found to have an influence on egg white lysozyme solubility, in that the enzyme dissolved at increasing temperatures and decreasing salt concentrations in sodium chloride solutions (Forsythe et al., 1999). The effect of sodium chloride and sucrose on the bactericidal activity of egg white lysozyme (HL80/6) as well as its synergy with glycine was investigated (Ibrahim et al., 1996). The bactericidal effect of the enzyme against *Escherichia coli* K12 was found to decrease with increasing sodium chloride and sucrose concentrations. Sodium chloride at 1% concentration was needed to suppress inhibition of *Staphylococcus aureus*. However, even at inhibitory doses of sodium chloride and sucrose, the enzyme exhibited good synergy with glycine with regard to its action against Gram-positive bacteria, and this according to the authors suggest a possible food preservative application in the industry.

Salt in fermented vegetables such as pickles, sauerkraut, soy sauce etc., plays a preservative role as well as a functional role controlling the rate of lactic acid bacterial growth and thereby the fermentation. Salt helps in creating an anaerobic condition in the fermentation vessels, thereby promoting the growth of lactic acid bacteria (Buckenhuskes, 1997). Salt is also added in canned vegetables mainly to provide flavor. About 0.5 to 2% salt is added to the hot blanching water during canning to make certain vegetables like peas and lima beans tender, and about 10 % is needed to soften the cucumber pickles for ease of packing (Kaufmann, 1960). Sodium chloride concentration had an influence on the flavor compounds produced by yeast in soy sauce (Sasaki, 1996). The glycoproteins produced by various yeast species under sodium chloride stress acted as cryoprotective agents (Breierova, 1997). Some of the flavor compounds were produced in the largest amounts at a sodium chloride concentration of 17-18%, which is the common amount used for soy sauce. Sodium chloride is rarely used as a preservative in fruit products. One product in which 6 to 8% brine is added at the intermediate stage before preserving with sugar is the raw material used for making succades (Lueck, 1980). However, research has shown that sodium chloride can have some indirect beneficial roles in the fruit industry. Polyphenol oxidase is the browning enzyme, which causes an undesirable color on many fruits. Sodium chloride has been effective in inhibiting this enzyme isolated from grapes at pH values less than 5.0 (Valero & Gracia-carmona, 1998). Polygalacturonase is an enzyme present in some fruits, which is used by the food industry in the extraction and clarification of fruit juices. This enzyme is thermo-labile and attempts have been made to increase its thermostability using various additives. Sodium chloride enhances the thermostability of this enzyme even at low pH values below the optimum for this enzyme (Devi & Rao, 1998).

In the case of meats, salt is mainly used in fermented sausages where it acts as a preservative as well as aids in the development of flavor and texture. With regard to other types of meats, ground meats have a concentration of 2-4%, bacon around 2.25%, hams

between 3 to 6% and corned beef about 6.25% of salt (Kaufmann, 1960). Salt lowers the water activity and in conjunction with nitrite can inhibit the growth of *Clostridium botulinum* (Terrell, 1983). Sodium chloride was found to show inhibitory effects against *C. botulinum* at a concentration of 4.5 to 4.8% (w/v) in wiener sausage even in the absence of nitrite (Hauschild, 1982). Concentrations lower than 4.5 % have been found ineffective against *C. botulinum*. Salt is also used in the curing and tanning of hides and skin. About 1 lb. of salt is added per lb. of hide and in curing, hides are immersed up to 24 h in saturated brine (Kaufmann, 1960). Salt is also used in the manufacture of animal by-products such as oleo stock, oleo oil and stearine. About 60 to 75 lbs of salt per 5000 lbs of the raw material is added at the melting stage and the final product contains about 2.5% salt added as a flavor enhancer and preservative (Kaufmann, 1960). Salt is also used in animal feeds, in fertilizers and weedicides, in soil stabilizers, in textile manufacture and in drilling oil wells.

VI. SAFETY AND TOLERANCE

The LD_{50} of sodium chloride when fed orally to fasted rats was determined to be 3.75 g/kg body weight (Boyd & Boyd, 1973). When administered to fasted rats over 100 days the LD_{50} was estimated to be 2.7 g/kg body weight and the respective value for non-fasted rats was 6.14 g/kg body weight (Lueck, 1980). A concentration of 2.8 to 5.6% sodium chloride given in the feed resulted in growth retardation and shortening of life-span of rats (Meneely et al., 1953). The LD_{50} for rats via oral route was estimated to be 3000 mg/kg, while via inhalation was >42 gm/m^3/1H1 (J. T. Baker, Material safety data sheet, 1996). The same reference cites LD_{50} for rabbits via skin to be >10 gm/kg and estimated it to be a mutagen and terratogen. For a detailed toxicity data and safety profile, refer to a food safety additives handbook.

About 1 to 3.3 g/day is the level of sodium that is considered safe as well as adequate for the body (Darby, 1980). Owing to its solubility in water, salt is excreted relatively easily and quickly from the body through the kidneys as well as the skin (in the form of sweat). Renal losses are about 6.0 to 12.5 g/day (Darby, 1980).

The effect of sodium chloride on cell division of rats was studied (Lugli & Lutz, 1999). Sodium chloride was given as a supplement at 2 and 4% concentrations in the feed for 4 weeks. This feed resulted in a significant stimulation of cell division in the stomach and liver with both concentrations of sodium chloride and in the bladder with 4% sodium chloride. Feed with ascorbic acid (2g/kg feed) or β-carotene (12.5 mg/kg feed) for 1 week before sodium chloride supplementation inhibited the stimulation of cell division.

VII. SUMMARY

Sodium chloride, commonly called table salt or salt, is a vital part of human life. Salt enhances the flavor of foods and plays a preservative as well as functional role in food processing. Apart from its use in the food industry, salt has uses in the agricultural and chemical industries as well as in water conditioning and transportation. Sodium chloride has been designated the fifth element and is abundantly available in nature. The production and recovery methods of sodium chloride are described. Two elemental substances, the cationic sodium and the anionic chloride, react to form the halide salt 'sodium chlo-

ride'. Sodium chloride occurs in the form of colorless transparent crystals or white crystalline powder, with a molecular weight of 58.44. To determine the salt content of a food product, the food or its ash is extracted in warm water and the sodium and chloride contents determined by appropriate techniques. The antimicrobial activity of salt can be both direct or indirect depending on the amount added and the purpose it serves. Since the amount of sodium chloride needed to be added to foods to prevent microbial growth is large and will cause an unacceptable taste, it is usually added in combination with other hurdles. The mechanism of inhibition of microorganisms by sodium chloride is mainly by lowering the water activity of the substrate. Studies have also indicated that sodium chloride could have a role in interfering with substrate utilization in microorganisms. The influence of sodium chloride alone as well as its interactive effects with other factors on various microorganisms such as *Staphylococcus aureus*, spore formers, *Escherichia coli* O157:H7, *Listeria monocytogenes* etc. in laboratory media as well as foods has been discussed in detail. The effect of sodium chloride on the heat resistance of microorganisms is discussed as well. The functional applications of sodium chloride in various products are discussed. Excessive sodium intake in humans has been linked to hypertension and the related cardiovascular problems and stroke. Hence there are many consumer concerns and the food industry is trying to minimize the salt content of food products.

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